

Evaluation of autologous serum eyedrops for the treatment of experimentally induced corneal alkali burns

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SUMMARY

The aim of this study was to evaluate the clinical, biochemical and histopathological efficacy of autologous serum eyedrops for the treatment of experimentally induced corneal alkali burns in rabbits. A total of 21 New Zealand white rabbits were used as experimental animals. The rabbits were randomly allocated to three equal groups. While the first group represented the control group (n=7), the right eyes of the rabbits in the second and third groups were exposed to alkali burns with 2 N NaOH after general anesthesia. After that, while autologous serum eyedrops were instilled four times a day into the eyes of rabbits in the second group for 21 days, non-dilute autologous serum was dropped into the eyes of rabbits in the third group in a similar fashion. Schirmer test, tear break-up time (TBUT), Rose bengal and fluorescein staining methods were performed on days one, seven, 14 and 21. At the end of the treatment period, all rabbits were euthanized. Aqueous humor and cornea samples were collected to obtain biochemical and histopathological data. Significant difference was observed between the third group and the second group ($P < 0,001$) at the end of 21 days. Malondialdehyde (MDA), reduced glutathione (GSH) concentrations, and catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) activities were analyzed in the cornea and aqueous humor. Histopathological, neovascularization, polymorphonuclear leukocytes (PMNL), and epithelial thickness were measured. Statistically, neovascularization and PMNL were found to be lower in the third group compared to the second group ($P < 0,05$). No significant difference was found between the groups regarding the epithelial thickness. Consequently, the results of the present study showed that topical application of undiluted autologous serum eyedrops can be useful in the treatment of corneal alkali burns.

Keywords: cornea, alkali wound, autolog serum eyedrops, rabbits

RÉSUMÉ

Evaluation d'un sérum autologue en gouttes dans le traitement brûlures alcalines de la cornée

Le but de cette étude était d'évaluer les effets cliniques, biochimiques et histopathologiques d'un collyre réalisé à partir d'un sérum autologue dans le traitement de brûlures alcalines de la cornée expérimentalement induites chez le lapin. Un total de 21 lapins blancs de Nouvelle-Zélande ont été répartis au hasard en trois groupes égaux. Le premier groupe représentait le groupe témoin (n = 7), l'œil droit des lapins des deuxième et troisième groupes ont été exposés à des brûlures alcalines avec NaOH 2 N après une anesthésie générale. Le collyre sérique autologue a été instillé quatre fois par jour pendant 21 jours dans les yeux de lapins du deuxième groupe alors que le sérum autologue non dilué a été appliqué d'une manière similaire dans les yeux des lapins du troisième groupe. Un test de Schirmer, le temps de pause lacrymal (TBUT), et des colorations au Rose Bengale et à la fluorescéine ont été effectuées les jours 1, 7, 14 et 21. A la fin de la période de traitement, tous les lapins ont été euthanasiés. L'humeur aqueuse et la cornée ont été recueillies pour analyses. Le malondialdéhyde (MDA), glutathion réduit (GSH), et les activités catalase (CAT), glutathion peroxydase (GSH-Px), superoxyde dismutase (SOD), et glutathion transférases (GST) ont été analysés dans la cornée et l'humeur aqueuse. La néovascularisation, l'infiltration par les polynucléaires neutrophiles (PMNL) et l'épaisseur de l'épithélium ont été mesurées. Des différences significatives ont été observées entre le deuxième et le troisième groupe ($P < 0,001$). Statistiquement, la néovascularisation et le PMNL étaient plus faible dans le troisième groupe ($P < 0,05$). Aucune différence significative n'a été trouvée entre groupes en ce qui concerne l'épaisseur de l'épithélium. Cette étude montre que l'application topique d'un sérum autologue non dilués peut être utile dans le traitement de brûlures alcalines de la cornée.

Mots-clés: œil, cornée, brûlures alcalines, sérum de autologue, collyre, lapin

Introduction

Alkalines cause more severe destruction in the cornea and conjunctiva than acids as they denature stromal collagen by rapidly penetrating tissues. Very severe ocular injury occurs following alkaline burns of the cornea. Lesions develop mainly in the corneal and conjunctival epithelium and stroma, endothelium, and episclera depending on the degree of penetration of the alkaline substance [15, 16, 37]. Corneal wound healing following alkali burns is a long term process with various complications such as neovascularization, melting and perforation, and dense opacity by scar formation [11, 29, 42].

Production of free radicals has been implicated in alkali burns. Free radicals derived from oxygen are known as reactive oxygen species (ROS) [4, 38]. Although alkali agents are independently toxic to the cornea, to a large extent their injury to living cells is also mediated by the generation of ROS [22, 27, 38]. To deal with the damage induced by ROS, the cornea possesses integrated enzymatic and non-enzymatic antioxidant and repair systems [9, 26, 34, 37]. An important corneal antioxidant enzyme, glutathione peroxidase (GSH-Px), is able to detoxify hydrogen peroxide and lipid peroxides in cells, and protect the cell protein and membrane against oxidation [6].



FIGURE 1: Group I, II and III postoperative 24 hours, 7th, 14th, 21th days view

Autologous serum eyedrops have been recommended for the treatment of several ocular surface disturbances such as persistent epithelium defect and keratoconjunctivitis sicca.

Biochemical and biomechanic contents of autologous serum are similar to those of normal lacrima. In addition to providing lubrication to the ocular surface, autologous serum like tears contain abundant growth factors and bactericidal compounds, and are also able to maintain cellular processes of epithelial repair. and to the reduce the risk of corneal contamination and infection [3, 8, 18, 23, 24, 29, 30, 36].

The purpose of the present study was to investigate the activity of autologous serum eyedrops in the treatment of experimentally-induced corneal ulcerations.

Materials and Methods

ANIMALS AND EXPERIMENTAL DESIGN

A total of 21 healthy New Zealand rabbits weighing 2.5-3 kg were used in the study. Rabbits were kept in special cages and maintained in the Experimental Research Center of Firat University during the study period. The rabbits were kept in standard laboratory conditions (24±3°C, 40-60% humidity, 12 hours of darkness, 12 hours of light) and were given standard pellet feed (Elaziğ Feed Factory, Turkey) and water *ad libitum*. Animals were randomly allocated to three groups of seven animals each. Group I was the positive control group

(no alkali burn and autologous serum eyedrops instillation), group II was the negative control group (alkaline burn and sterile saline solution instillation), termed the: AB+SS group, and finally group III was the treated group (alkaline burn and autologous serum eyedrops, termed the AB+AS group).

ANESTHESIA TECHNIQUE

Anesthesia was applied to groups II and III by intramuscular injection of 5 mg/kg xylazine hydrochloride (Rompun, 23.32 mg/ml, Bayer, İstanbul, Turkey) followed by 35 mg/kg ketamine hydrochloride (Ketalar, 50mg/ml, Eczacıbaşı, İstanbul, Turkey). Corneal analgesia was provided by dropping 0.5% proparacaine hydrochloride (Alcaine ophtalmic solution 0.5%, Alcon Laboratories, İstanbul, Turkey) on to the eye before creating the corneal lesion.

CREATING CORNEAL ALKALINE BURN

After the right eyes of groups II and III rabbits under general anesthesia were maintained opened with a blepharostat, a 6 mm in diameter filter paper was immersed into 2 N NaOH and placed in the center of the cornea for 1 minute (figure 1). The cornea was then flushed with sterile saline solution for 2 minutes.



FIGURE 2: no lesion (Group I)

PRODUCTION OF AUTOLOGOUS SERUM EYEDROPS AND APPLICATIONS

After surgical preparation of the outer surface of one ear, 8 to 10 ml of blood was withdrawn from vena auricularis with a vacutainer. The blood samples were centrifuged at 4000 rpm for 10 min to obtain autologous serum. Obtained sera were stored at +4 °C until required. Separated autologous serums are transferred to 0.5 ml sterile non-light transparent droppers. Prepared droppers are frozen in -20 degrees. Droppers are changed every two days and droppers are kept in refrigerators in +4 degrees until the end of their expiry dates with their mouths closed.

Three drops of non-diluted autologous serum were dropped into the eyes of rabbits in groups I and III, while those in group II received sterile saline. All administrations were made four times a day, for 21 days.

CLINICAL EXAMINATION

Before the study all rabbits underwent a complete ophthalmic examination, including indirect ophthalmoscopy, slit lamp biomicroscopy, Schirmer's tear test and fluorescein staining.

Low magnification ophthalmic examination, slit lamp biomicroscopy, and the fluorescein and Rose bengal dye tests

were performed on the first, seventh, 14th and 21st days of the observation period. The wound margin was outlined directly with fluorescein solution, and the eyes were photographed by a digital camera on the first, seventh, 14th and 21st days. In addition clinical outcome was monitored and documented every day by corneal opacity, duration of blepharospasm, corneal vascularization and duration of ocular discharge.

The ocular signs were evaluated as photophobia/blepharospasm, ocular discharge, conjunctival congestion and hyperemia, chemosis, and corneal transparency/opacification, edema, neovascularization and pigmentation. The changes in the aqueous humor were examined in terms of transparency, hemorrhage and debris. The severity of ulceration was scored as follows; grade 0: no ulceration, grade 1: epithelial defect or lesion reaching the superficial stroma, grade 2: lesions reaching the midstroma, grade 3: lesions reaching the deepest layer of the stroma, grade 4: lesions reaching the descemet membrane, grade 5: corneal perforation [4, 10]. The Schirmer tear test was performed using standard sterile tear-measurement strips (Optitech, Tarun Enterprises, India) kept in the lower fornix for 60 seconds (Table I).

For the tear break-up time (TBUT) 1 µl drop from 1% of fluorescein sodium was instilled into the right eyes of each subject. Once the blink occurred the eye lids were kept open gently with fingers and TBUT was assessed under slit-lamp bio microscope using cobalt blue filter. The time when the point breaks first became visible was recorded as BUT (Table II).

This measurement was made on the first, seventh, 14th and 21st days in all groups. The ocular surface was examined after application, with a drop of 1% fluorescein and using the blue cobalt light under the slit lamp biomicroscope. Afterwards, the eye was flushed with saline and one drop of Rose bengal dye was instilled before examination of the ocular surface under red light illumination.

Rose bengal (Institut Pourquier, Montpellier, France) staining of the ocular surface was scored according to the criteria proposed. The fluorescein staining (Madhu Instruments, New Delhi) was scored according to the protocol described [3, 19, 23]. Briefly, the cornea was divided

Groups	Time points (day)				
	0	1	7	14	21
Group I	16.14±1.03	15.57 ±1.13 ^A	16.43±0.95 ^A	17.43±0.57 ^A	17.43±0.95 ^A
Group II	15.43±1.95	10.00±1.13 ^B	8.14±0.77 ^B	7.14±1.12 ^B	7.57±0.69 ^B
Group III	15.43±1.36	8.86±0.51 ^B	13.43±1.00 ^A	12.71±1.02 ^C	12.86±1.12 ^C
P ²	>0.05	< 0.001	< 0.001	< 0.001	< 0.001

¹A general linear model was used to determine the differences between groups, time, and interaction groups-time; P values were <0.001, >0.05 and <0.01, respectively.

²Two-way analysis of variance for repeated measures and post hoc Tukey were used to determine the differences between groups and between time points. Different superscripts within the same column demonstrate significant differences.

TABLE I: Mean ± SEM values of Schirmer tear test in different time points of control, AB+SS and AB+AS groups (n=7).¹

into three equal areas of upper, middle, and inferior corneal compartments. Each compartment was graded on a scale of 0 (no staining) to 3 points (intense staining).

BIOCHEMICAL EXAMINATION

In order to determine the course of healing in all groups in terms of biochemical and histopathological aspects, all rabbits were euthanized after general anesthesia at the end of the observation period (the 21st day). The cornea and aqueous humor were removed rapidly and utilized for histological and biochemical analyses, as described below.

The tissue samples were mixed with 1.15% KCl (pH 7.4) at the 1:10 ratio (weight/volume) then homogenized with a Potter-Elvehjem glass-glass homogenizator onto fragmented ice. The homogenates were centrifuged at 18,000×g (+4°C) for 30 min to determine malondialdehyde (MDA), reduced glutathione (GSH) concentrations, and catalase (CAT), superoxide dismutase (SOD) activities and at 25,000×g for 50 min to determine glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) activities. In addition, the protein concentrations were determined on the obtained supernatant fractions.

The concentration of MDA as a marker of lipid peroxidation were determined according to the method of PLACER *et al.*, [28] based on the reaction with thiobarbituric acid. The formed MDA created a pink complex with thiobarbituric acid (TBA) and the absorbance was read at 532 nm. The tissue MDA content was expressed as nmol/g tissue.

Tissue GSH concentration was measured by an assay using the dithionitrobenzoic acid recycling method described by ELLMAN [12], and was expressed as μmol/ml.

CAT activity was determined according to the method of AEBI [1], and was expressed as k/g protein. The degradation rate of H₂O₂ by CAT was spectrophotometrically measured by means of the fact that H₂O₂ absorbs light at the 240 nm wavelength and was expressed as k/g protein.

GSH-Px activity was determined using the method of BEUTLER [5], which records the disappearance of NADPH at 340 nm and was expressed as U/g protein.

SOD activity was determined using the method of SUN *et al.*, [35]. The SOD enzyme activity measurement is based on the nitrobluetetrazolium (NBT) degradation by the superoxide radical, which was produced with the xanthine-xanthine oxidase system. The formazan obtained at the end of the reactions exhibits a blue color and maximally absorbs at 560 nm.

GST activity was determined spectrophotometrically by measuring the 1-(S-glutathionyl)-2,4 dinitrobenzene compound forming as a result of the conjugation of GSH and 1-chloro-2,4-dinitro benzene (CDNB) at 340 nm. The GST activity was calculated as U/mg protein [17].

Protein concentrations were measured according to LOWRY *et al.*, [25]. The alkaline copper tartaric reagent (potassium tartrate, sodium carbonate, copper sulfate, sodium hydrochloride) reagent forms a complex with peptide bonds. When phenol reagent is added to the mixture treated with copper, a purple-blue color is formed. The color intensity was read at 650 nm.

HISTOPATHOLOGICAL EXAMINATION

One part of cornea was used for histopathological analyses. Fixed corneal tissues in 10% neutral formalin were dehydrated through graded concentrations of ethanol embedded in paraffin wax, sectioned at 5μ thicknesses and stained with hematoxylin and eosin staining. Each sample was examined for neovascularization, polymorphonuclear leukocytes (PMNL) and epithelial thicknesses. Seven microscopic fields were randomly selected. Neovascularization and the existence of PMNL were classified as none (1), few (2), middle (2) and severe (3). Epithelial thickness was classified as none (0), 1-2 layers-(1), 3 layers-(2), 4-5 layers-(3).

STATISTICAL ANALYSIS

All values are presented as means±SEM. P < 0.05 was considered as significant. General Linear Model (GLM) procedure and two-way analysis of variance for repeated

Groups	Time points (day)				
	0	1	7	14	21
Group I	10.00±0.82	11.00±0.65 ^A	11.43±0.53 ^A	11.43±0.53 ^A	12.14±0.80 ^A
Group II	10.14±1.06 ^a	4.71±0.42 ^{Bb}	5.86±0.51 ^{Bb}	4.86±0.26 ^{Bb}	4.14±0.51 ^{Bb}
Group III	9.71±0.84 ^a	5.86±0.34 ^{Bb}	7.29±0.61 ^{Bc}	7.71±0.42 ^{Cd}	6.86±0.51 ^{Ce}
P ²	>0.05	<0.001	<0.001	<0.001	<0.001

¹A general linear model was used to determine the differences between groups, time, and interaction groups-time; P values were <0.001, <0.01 and <0.001, respectively.

²Two-way analysis of variance for repeated measures and post hoc Tukey were used to determine the differences between groups and between time points. Different superscripts within the same column or a same row demonstrate significant differences.

TABLE II: Mean ± SEM values of TBUT in different time points of control, AB+SS and AB+AS groups (n=7).¹

measures were used to determine the differences between groups and between time points with respect to Schymer, TBUT, Rose bengal and Fluorescein parameters. All biochemical (MDA, GSH, CAT, GSH-Px, SOD and GST) and histopathological (Neovascularization, PMNL and Epithelial Thickness) parameters were compared by one-way analysis of variance (ANOVA) and post-hoc Duncan test. SPSS program (15.0, Chicago, IL, USA) was used for statistical analysis.

Results

CLINICAL FINDINGS

Corneal edema and vascularization started immediately after burning. The corneal stroma became very opaque within the 24 h following the corneal injury. Within three days following alkali burn, the rabbits from groups II and III showed marked blepharospasm (eyes were semi-closed) with corneal epithelial defect and ulcer formation. The healing signs were seen in the corneal opacity of rabbits in the autogenous serum group except for two animals, but no change was observed in control group at the end of day 21 (figure 1). The healing of the corneal wounds was determined by Schirmer and TBUT test, by Fluorescein and Rose bengal staining methods and by biochemical and histopathological examinations as demonstrated in tables (I, II, III, IV).

BIOCHEMICAL FINDINGS

MDA levels increased more in the cornea and aqueous humor in group II in comparison with the control group ($P < 0.05$). However, cornea and humor MDA levels normalized ($P < 0.05$) in the group treated with AS compared to group II ($P < 0.05$).

GSH levels decreased in the cornea and humor tissues in group II in comparison with the control group ($P < 0.05$). The humor GSH levels were normalized in treatment with AS.

CAT activity was found to be decreased ($P < 0.05$) in the cornea tissue of the animals compared with the control group in group II ($P < 0.05$), but no such effect was seen in group III.

While GST activity was found to be increased ($P < 0.05$) in the cornea and humor tissues of the animals compared with the control group in group II ($P < 0.05$), it was found to be decreased in the humor aqueous in group III.

GSH-Px activity was found to be decreased ($P < 0.05$) in the cornea of the animals compared with the control group in group II ($P < 0.05$). There was no significant difference in GSH-Px activity in tissues in group III in comparison with group II.

Groups	Time points (day)				
	0	1	7	14	21
Group I	0.00±0.00	0.00±0.00 ^A	0.00±0.00 ^A	0.00±0.00 ^A	0.00±0.00 ^A
Group II	0.00±0.00 ^a	3.85±0.14 ^{Bb}	3.71±0.18 ^{Bb}	3.85±0.14 ^{Bb}	3.42±0.20 ^{Bb}
Group III	0.00±0.00 ^a	4.00±0.00 ^{Bb}	3.28±0.35 ^{Bb}	2.28±0.18 ^{Cc}	1.85±0.14 ^{Cc}
<i>P</i> ²	>0.05	< 0.001	< 0.001	< 0.001	< 0.001

¹A general linear model was used to determine the differences between groups, time, and interaction groups-time; *P* values were <0.001, <0.01 and <0.001, respectively.

²Two-way analysis of variance for repeated measures and post hoc Tukey were used to determine the differences between groups and between time points. Different superscripts within the same column or a same row demonstrate significant differences.

TABLE III: Mean ± SEM values of Rose bengal at different time points of control, AB+SS and AB+AS groups (n=7).¹

Groups	Time points (day)				
	0	1	7	14	21
Group I	0.00±0.00	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Group II	0.00±0.00	3.00±0.00 ^b	3.00±0.00 ^b	3.00±0.00 ^b	3.00±0.00 ^b
Group III	0.00±0.00	2.71±0.18 ^b	1.85±0.14 ^c	1.71±0.18 ^c	1.42±0.20 ^c
<i>P</i> ²	>0.05	< 0.001	< 0.001	< 0.001	< 0.001

¹A general linear model was used to determine the differences between groups, time, and interaction groups-time; *P* values were <0.001, >0.05 and <0.001, respectively.

²Two-way analysis of variance for repeated measures and post hoc Tukey were used to determine the differences between groups and between time points. Different superscripts within the same column demonstrate significant differences.

TABLE IV: Mean ± SEM values of Fluorescein in different time points of control, AB+SS and AB+AS groups (n=7).¹

SOD activity was found to be decreased in the cornea tissues of the animals compared with the control group in group II ($P < 0.05$), but the inhibition of cornea SOD activities were normalized in treatment with AS.

GST activity was found to be increased in the cornea and humor tissues of the animals compared with the control group in group II ($P < 0.05$), but GST activities were normalized in treatment with AS ($P < 0.05$).

HISTOPATHOLOGICAL FINDINGS

Histopathologically, no lesion was found in Group I (figure 2). Neovascularization and PMNL infiltrations were statistically ($P < 0.05$). higher in group II than group III (Table VI and figures 3, 4). However, neovascularization and cell infiltrations in one rabbit of group III were equal in level to group II. All groups had four to five cell layers and no statistically significant differences were determined between the groups in terms of epithelial thickness (Table VI). Stromal edema was observed in six of seven rabbits in group II but only in two of seven in group III.

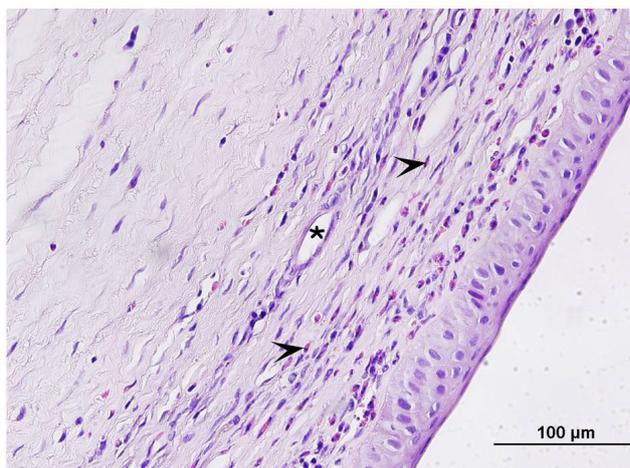


FIGURE 4: Groups III. Neovascularization (*), and PMNL cells (arrowheads)

Discussion

Alkali burns of the cornea are among the most serious ocular injuries. Secondary complications such as infection, ulceration, perforation, neovascularization, and opacification can develop and threaten patients' vision. Early and effective treatment to enhance and support the biological healing process by various medical means is thus necessary. [11, 15, 16, 30, 42].

Various treatment modalities have been evaluated including amniotic membrane grafting [10, 32, 33], subconjunctival injection of platelet-rich plasma [14], topical application of acetylcysteine [13, 21], umbilical cord serum, honey, propolis extract, and Na-hyaluronan [4, 26]. Many clinical and experimental studies have reported the therapeutic use of autologous serum eyedrops. It has been shown that autologous serum supplies better protection of the corneal epithelium, and stimulates corneal epithelial migration, providing accelerated epithelial healing and

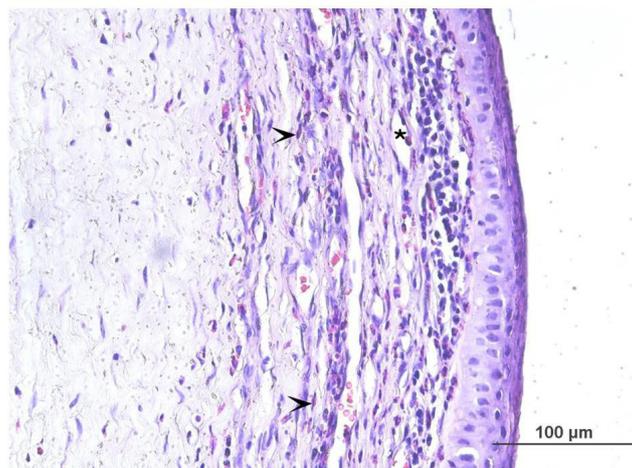


FIGURE 3: Groups II. Neovascularization (*), and PMNL cells (arrowheads)

Parameters	Cornea			Humor Aquous		
	Group I	Group II	Group III	Group I	Group II	Group III
MDA (nmol/g tissue)	0,33±0,01 ^c	0,43±0,01 ^a	0,38±0,01 ^b	0,23±0,02 ^b	0,31±0,01 ^a	0,26±0,01 ^b
GSH (µmol/ml)	13,68±1,13 ^a	8,12±0,31 ^b	8,62±0,50 ^b	13,62±1,01 ^a	9,30±0,58 ^b	15,84±1,68 ^a
CAT (k/g protein)	11,05±0,19 ^b	5,01 ± 0,91 ^a	15,94 ± 3,10 ^a	0,33±0,06 ^{ab}	0,18±0,04 ^b	0,65±0,16 ^a
GSH-Px (U/g protein)	9,86±0,26 ^a	3,33±0,62 ^b	8,90±2,76 ^{ab}	0,08±0,01	0,05±0,02	0,62±0,33
SOD (U/mg protein)	0,90±0,03 ^a	0,57±0,04 ^b	0,86±0,09 ^a	4,22±0,29 ^{ab}	2,89±0,07 ^b	6,94±1,87 ^a
GST (U/mg protein)	6,27±0,82 ^b	11,97±1,13 ^a	8,72±0,65 ^b	0,11±0,02 ^b	1,82±0,35 ^a	0,10±0,04 ^b

^{a-c}: Different superscripts within the same line demonstrate significant differences ($P < 0.05$).

TABLE V: Mean ± SEM values of biochemical parameters in corneal tissue and humor aquous of control, AB+SS and AB+AS groups (21th days post surgery).

reducing other secondary ocular surface problems. Our results also suggest that undiluted autologous serum enhanced the early epithelial healing and provided better maintenance of the resurfaced epithelium [11].

In the treatment of aqueous-tear-deficient dry-eye disease, HOCSAN *et al.*, [19] and SALMAN *et al.*, [31] used undiluted autologous serum, 20% diluted autologous serum, physiologic saline solution and preservative free artificial tear drops topically four times a day, and observed the best results with the undiluted autologous serum eyedrops.

In the early experimental study of SHAHRIARI *et al.*, [33], a positive effect on the epithelial healing rate was not observed with application of 20% diluted autologous serum eyedrops in alkaline burned corneas when compared with the control group. We observed an increase in epithelial healing rates when undiluted serum eyedrops were used in comparison to physiological saline, based on high concentrations of essential factors which supports the proliferation of corneal epithelial cells. In our study, no corneal perforation was also observed in the autologous serum treatment group suggesting that this treatment is able to prevent stromal lysis.

Some authors did not find statistically significant differences between this treatment and conventional therapy [7, 21, 33]. FREITAS *et al.*, [13] reported that the autologous serum did not alter the healing process kinetics of dog corneas that suffered the caustic effects of 3 M sodium hydroxide compared to corneas that received 0.90% balanced saline solution. Likewise in the same study, autologous serum had no effect on corneal alkaline burn ulcer healing when compared with the control group for clinical and histopathological factors. [4, 21]. In our study there was only mild corneal infiltration by PMNL and neovascularization in histopathological examination of the autologous serum treatment group. This suggests that the undiluted autologous serum has the anti-inflammatory component comparable to that of the control group.

The mechanism of action of autolog serum relies on its content in proteins, such as fibronectin, and albumin as epidermal growth factors necessary for epithelial regeneration after alkali injury of the cornea [20, 30, 39, 40]. Epidermal growth factors have been shown to be beneficial in promoting epithelial migration in experimental as well as

human studies of corneal alkali injury [7, 42].

Chemical eye injury is accompanied by a decrease in antioxidant protection systems as well as activation of the lipid peroxidation. In a study by ALIO *et al.*, [2] antioxidant topical treatment with dimethylthiourea was found to be efficient in reducing inflammatory reactions during acute corneal inflammation. Increased MDA levels, the final product of lipid peroxidation, and decreased SOD activities, an enzymatic scavenger of the superoxide free radical, have been reported in the aqueous humor after the severe alkali burn of the cornea [41]. Results of our study are consistent with these data.

In this study, the antioxidant activities in the aqueous humour and cornea were found to be higher in the eyes treated with autologous serum eyedrops than in those of the negative control group. This denotes that autologous serum can increase the antioxidant capacity in the ocular tissues of rabbits. In addition the use of autologous serum following cornea alkaline burn significantly decreased the MDA levels in the aqueous humor and cornea in comparison to the control group [9, 14]. GSH-Px is an important corneal defense enzyme for protecting against hydrogen peroxides and lipid peroxides [34]. SALMAN *et al.*, [31] reported that after alkali burns the corneal GSH-Px activities were reduced. In our study, the GSH-Px activity in groups treated with autologous serum was higher than that of the control group. Our results suggest that, even after at mild alkali burn, the corneal scavenging systems for hydrogen peroxides and lipid peroxides were reduced. Thus, the cornea becomes more susceptible to the harmful effects of reactive oxygen radicals after the alkali burn. Our results are supported by those of the study by OZTURK *et al.*, [26].

The results of this study showed that the use of local autologous serum eyedrops in corneal alkali burn is a simple and economical treatment, free of undesirable side effects. It is a safe and easily producible material containing several mediators such as growth factors, which are needed in wound healing. Autologous serum seems to be a promising agent for clinical use in the cornea alkaline wound healing process.

Groups	Neovascularization	PMNL	Epitelyal thickness
Group I	0.00±0.00 ^a	0.00±0.00 ^a	3.00±0.00 ^a
Group II	2.71±0.18 ^c	2.83±0.16 ^c	3.00±0.00 ^a
Group III	1.85±0.24 ^b	2.16±0.16 ^b	3.00±0.00 ^a
Significance	P < 0.05	P < 0.05	NS

^{a-c} : Different superscripts within the same column demonstrate significant differences NS: Not significant.

^aP < 0.05 versus Group II and Group III, ^bP < 0.05 versus Group I and Group III, ^c P < 0.05 versus Group I and Group II.

TABLE VI: Mean ± SEM values of histopathological findings in corneal tissue of control, AB+SS and AB+AS (n=7)

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